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Barn *vs.* free-range chickens: Differences in their diets determined by stable isotopes

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ABSTRACT

We compared $\delta^{13}C$ and $\delta^{15}N$ ratios of barn-raised and free-range chickens to determine if differences in their diets were reflected in the stable isotope composition of their tissues. We conducted a 120-day feeding trial with *Caipirinha* birds fed a corn–soybean based diet, milled-corn diet and free-range diet. Additionally, we analysed the stable isotope composition of barn-raised chickens bought in grocery stores and free-range homegrown chickens. In the feeding trials, the $\delta^{13}C$ and $\delta^{15}N$ values of the barn-raised corn–soybean-fed *Caipirinha* chickens did not change with age, and their stable isotope composition reflected the composition of their diet. The $\delta^{13}C$ and $\delta^{15}N$ values of barn-raised corn-fed and free-range *Caipirinha* chickens changed with age toward a diet reflecting a predominance of C_4 carbon. The main difference between the free-range and the barn-raised chickens was the significantly higher $\delta^{15}N$ of the former in relation to the latter, probably due to ingestion of animal protein.

1. Introduction

The growth in chicken production began mainly in the early 1970s with the initial recommendations that chicken meat was healthier than beef, and also due to the fact that chicken meat is less expensive than beef and pork (Bolis, 2002; Roberts, 2008). This significant increase in poultry production and consumption occurring in recent decades was only possible due to a series of changes in the poultry industry. These changes included, among others, changes in feed composition based on grains such as corn and soybean and some source of animal protein, and genetic improvements to increase feeding efficiency and the amount of breast meat (Roberts, 2008).

Regarding diet availability to chickens, there is on one side what are conventionally referred to as barn-raised chickens, in which the main nutritional characteristic is that this diet is provided exclusively by the producer; on the other side, there are the so-called free-range chickens, which roam freely through meadows and mimic their original foraging dietary habits, eating not only grass but also earthworms from the soil (Fanatico, 2006; Sossidou, Dal Bosco, Elson, & Fontes, 2011; Zanusso & Dionello, 2003). The demand for such a product has been increasing in recent decades (Castellini, Mugnai, & Dal Bosco, 2002; Fanatico, Pillai, Emmert, & Owens, 2007).

Stable isotopic ratios of carbon and nitrogen have been largely used to infer diet sources of animals (Kelly, 2000; Newsome, del Rio, Bearhop, & Phillips, 2007). The first rationale justifying the use of stable isotopes for studying animal nutrition is related to the fact that carbon and nitrogen isotopes show relatively few and predictable changes when the atoms of these two elements pass through the food chain (Bearhop, Adams, Waldron, Fuller, & Macleod, 2004; Newsome et al., 2007). Therefore, the stable carbon and nitrogen isotopic composition of an animal will reflect approximately the same composition as their feed (e.g., Nardoto et al., 2006; Rogers, 2009). There is an extensive body of literature showing the usefulness of this technique, based on the pioneering work of DeNiro and Epstein (1978, 1981) and Tieszen, Hein, Qvortrup, Troughton, and Imbamba (1979), among others.

Stable isotopes have been especially useful in nutrition ecology of wild animals (Newsome et al., 2007). There is also a growing use of stable isotopes to investigate livestock in terms of authenticity and nutrition studies (e.g., Bahar et al., 2009; Guo, Wei, Pan, & Li, 2010; Harrison et al., 2010, 2011; Heaton, Kelly, Hoogewerff, & Woolfe, 2008; Osorio, Moloney, Schmidt, & Monahan, 2011; Schmidt et al., 2005). Several studies involving livestock are based on changing

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the original diet for another with different isotopic composition to track changes of isotopic composition of tissues over time (e.g., Ayliffe et al., 2004; Bahar et al., 2005; Harrison et al., 2011; West et al., 2004). Specifically in poultry, stable isotopic composition has been used to determine turnover time in tissues (Cruz et al., 2005; Hobson & Clark, 1992); nutrient routing (Cruz et al., 2004); to track the presence of animal protein in commercial poultry rations (Carrijo et al., 2006; Denadai, 2008; Mori et al., 2007), to detect the presence of corn in poultry diet (Rhodes et al., 2010), and to differentiate eggs laid by hens under different growth systems (Rogers, 2009). The latter was the only study designed to investigate differences between barn-raised and free-range chickens, and it was specifically designed for eggs and not for meat. Therefore, there is a need for studies that aim to investigate whether it is possible to differentiate barn-raised from free-range chickens by the use of stable isotopes. This is especially important because free-range chickens usually have a higher price than barn-raised chickens.

The main objective of this study was to investigate temporal changes in the isotopic composition of chickens grown under controlled conditions receiving two different diets. One diet was a conventional grain-based ration used in commercial barn-raised chicken plants, and the other diet was typical of free-range chickens in Brazil, which is a mixture of corn, grass and earthworms from the soil. We further compared the stable isotopic composition of barn-raised chickens bought in grocery stores, produced by 15 different companies, with 27 homegrown free-range chickens obtained from local households.

2. Material and methods

2.1. Feeding trials

The *Caipirinha* is a slow-growing chicken developed by the Genetic Department of the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ) and its main characteristics are its resilience and adaptability as a free-range bird, especially developed for homegrown conditions. Seventy-five *Caipirinha* broilers received the same corn and soybean starter feed *ad libitum* for the first 28 days (Table 1). After this period, broilers were divided into three groups of 25 birds each and were fed *ad libitum* with three different diets. One group continued receiving a final corn and soy-based feed (*Caipirinha*-barn-raised corn–soybean-fed; Table 1), a second group was allowed free access to grass pasture areas and also received milled corn (*Caipirinha*-free-range), and, finally, a third cohort received only milled corn (*Caipirinha*-barn-raised corn-fed). Individuals for each diet-treatment were kept apart and those allowed to pasture had free access to grass areas.

At 28, 60, 90, and 120 days of age, five individuals randomly selected from each treatment were slaughtered and the breast muscle of each bird was analysed for carbon and nitrogen stable isotopes.

Samples of each type of diet (starter and final), five samples of grasses and surface soil (0–10 cm) randomly sampled from the pasture plots were also collected for stable isotope analysis.

Table 1
Composition of the corn–soybeans rations used in feeding trials.

Parameter	Start – 28 days	Finish – 28 to 120 days
Metabolisable energy (kcal kg^{-1})	2950	3050
Crude protein (%)	21.0	18.0
Crude fibre (%)	4.50	4.50
Ether extract (%)	3.00	3.50
Calcium (%)	1.20	1.20
Phosphorus (%)	0.65	0.60
Lysine (%)	1.19	0.97
Methionine + cystine (%)	0.89	0.76
Linoleic acid (%)	1.80	2.00
Xanthophylls (ppm)	9.50	1.10

2.2. Barn-raised and free-range homegrown chickens

We analysed the breast muscle of 32 barn-raised chickens bought in grocery stores, produced by 15 different companies, and 27 homegrown free-range obtained from local households in Brazil. Information about the diet composition of all barn-raised birds was provided on all commercial brand labels, being mostly composed of grains. However, the proportion of each grain was not divulged. The main grains of these feeds were milled corn, milled sorghum, wheat meal, soybean meal, cotton meal, and pearl rice; and the main animal protein sources were: bone meal, offal meal, fish meal, and feather meal. It is important to mention that we did not use these diets to feed chickens in our feeding trials. The household birds had free access to grass areas, and rations of milled corn and leftovers from homemade meals were also offered to them, such as cooked rice and beans, and greens from salads, such as lettuce, kale, arugula, etc.

2.3. Isotopic analyses

All chicken breasts were oven-dried at 65 °C until constant weight and then ground to a fine powder. We did not extract lipids from our samples because breast muscles of Brazilian chickens have a very low lipid content, varying from 0.5% to 1.5% (Assis et al., 2010). Although there are several studies showing that lipids tend to have a lower $\delta^{13}C$ ratio than tissues with low lipid content (Bahar et al., 2009), this amount of lipids would probably not affect our results.

Soil and grass samples were air-dried, sieved using a 2-mm mesh and homogenised. A smaller sub-sample was collected, handpicked to remove fine roots and other debris and then ground in a mortar and pestle. A 1.5–2 mg sub-sample of ground chicken and leaf material or 15–20 mg sub-sample of ground soil were placed and sealed in a tin capsule and loaded into a ThermoQuest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT; San Jose, CA) in line with an Elemental Analyser (Model 1110; Carlo Erba, Milan, Italy). Stable isotope ratios of C and N were measured relative to recognised international standards. Internal working standards (sugarcane leaves and tropical surface soil) were included in every run, as a standard laboratory procedure. Stable isotope values are reported in "delta" notation, as δ values in parts per thousand (‰), so that $\delta‰ = (R\ \text{sample}/R\ \text{standard} - 1) \times 1000$, in which R is the molar ratio of the rare to abundant isotope ($^{15}N/^{14}N$; $^{13}C/^{12}C$) in the sample and the standard. The precision of the measurements was ±0.3%, 0.1%, 0.3‰ and 0.5‰ for C, N, $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.4. Statistical analyses

The Shapiro–Wilk test was used to test the normality of the data. As the data followed a normal distribution, the analyses were performed using parametric tests (ANOVA). A *post hoc* Tukey test was used to assess differences between stable isotopic compositions of *Caipirinha* chicken fed with different diets. All statistical analyses were performed using the software STATISTICA, Version 9.0 for Windows (StatSoft, Tulsa, OK, 2010). Differences at the 0.05 level were reported as significant.

In order to estimate the turnover rate of breast tissue, we used the following equation:

$$\delta_t = \delta_n + (\delta_0 - \delta_n) * e^{-(ct)} \quad (1)$$

where δ is the $\delta^{13}C$ or $\delta^{15}N$ values of the breast muscle at time t after the diet change; δ_0 is the initial $\delta^{13}C$ or $\delta^{15}N$ values of the breast muscle before the diet change at time $t = 28$ days; δ_n is the $\delta^{13}C$ or $\delta^{15}N$ values of the breast muscle in equilibrium with the new diet; c is the total turnover; and t is the time in days since the start of the